ON THE GERMINATION OF THE POLLEN GRAINS OF APPLE AND OTHER FRUIT TREES

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The following observations were made at Dublin, Ireland, in 1913, and were of a preliminary nature only. It was hoped to base a much more complete series of experiments in 1914 on the information gained in the previous year. Owing to unforeseen circumstances this was not possible, and as no opportunity for continuing the investigation is likely to arise in the near future, the results so far gained are given in the hope that others may find them useful for comparison.

There is much connected with the question of pollination that is still obscure. For instance, it is known that a russet apple produces seeds plentifully, while a Newton pippin contains only a few seeds. In both cases the fruit is equally well developed. Is there any relation between the number of seeds in the fruit and the number of pollen grains that reach the stigma? Presumably there is, but one would imagine that the chances of pollination are the same in both varieties.

Failure of an individual flower to produce fruit may be due to a variety of causes. The flower may not have been pollinated; it may have been pollinated from another flower of the same variety; it may not have been sufficiently pollinated, that is, the stigma may have received only a small number of pollen grains; it may have been pollinated too late, the stigma having ceased to be receptive; the pollen grains may have been rudimentary and incapable of germination; the temperature may have been too low for the pollen grains to germinate; or the ovary or stigma may have been injured by frost, either before the flower opened or subsequently. It seems obvious that a full understanding of all these matters is of fundamental importance to all engaged in fruit growing or seed production.

The following observations relate to the germination of the pollen grains in a cane sugar solution, their behavior when placed

on the stigma of the same or another variety not being investigated. Attempts were made to answer the following questions: (1) what strength of sugar solution gives the most rapid germination; (2) how is the germination of the pollen grains affected by temperature; (3) what is the rate of growth of the pollen tube; (4) how long, under the most favorable conditions, do the pollen grains retain their vitality?

Most of the following facts and figures relate to the apple, but a few observations were made also on other fruits.

Apple

The pollen grains when dry were ellipsoidal, and comparatively little variation in size was found in the varieties examined. While some were longer and narrower, and others shorter and broader than the figures given, the average dimensions were as follows: Bramley's seedling $45\times24\ \mu$, Wyken pippin $45\times24\ \mu$, Duchess of Oldenburgh $42\times27\ \mu$, Warner's King $46.5\times25.5\ \mu$, Cox's orange pippin $45\times25.5\ \mu$, Nelson $43.5\times24\ \mu$, Bismarck $45\times24\ \mu$. When the pollen grains were wet, they swelled up so as to become more globular in shape. For instance, the pollen grains of Bramley's seedling when wet measured $45\times39\ \mu$. This preliminary swelling appears to be the first stage in the germination of the pollen grain.

SERIES I

For the first set of observations, solutions of cane sugar of 5, 10, 15, 20, 25, 30, 40, and 50 per cent strength were made. These were preserved in stoppered bottles for subsequent use, but it was found that after taking out a drop of the solution with a glass rod and replacing the cork, an abundant growth of mycelium of *Penicillium* developed in the bottles. Accordingly a fresh stock of solutions had to be made, and after taking a drop out of any of these the bottle was invariably brought to the boiling point. It was found that by so doing the sugar solutions could be kept indefinitely.

As a general rule, dry pollen grains were taken from an anther which had recently dehisced, and these were dusted on to a clean glass slide. A drop of the sugar solution was placed on the pollen

grains, and the drop was stirred with needles so as to make sure that the surface of the pollen grains was wet and that they were distributed uniformly through the solution. It was found in later experiments that the pollen grains germinated much more rapidly if a very thin layer of liquid was spread on the slide than if a large hemispherical drop was used, the reason being presumably that oxygen was more readily obtained by the pollen grains in the former case. Cover glasses were not used, as they prevented germination except along the edges. The prepared slides were then placed on the surface of damp blotting paper in Petri dishes and the cover replaced. In other cases a larger number of slides was placed on blotting paper in a glass dish with flat bottom and a glass plate was used as a cover. The presence of the damp blotting paper served to diminish evaporation of the sugar solution.

On May 6, 1913, two sets of cultures were started at 4:00 P.M. One was kept in the laboratory, the temperature of which at this time was 14°.5 C., the other being placed on the window ledge outside, which faced toward the north, so that direct sunshine could not reach it. The temperature outside at the time of starting the culture was not observed. The cultures were examined daily during the next 3 days. It was found impracticable to keep them going longer than 3 days, owing to the occurrence of *Penicillium*. The strengths of sugar solution, as stated above, were: (*a*) 5, (*b*) 10, (*c*) 15, (*d*) 20, (*e*) 25, (*f*) 30, (*g*) 40, and (*h*) 50 per cent. The results were as follows:

A_1.—Inside cultures: 1:00 P.M., May 7; temperature 13° C.: (*a*) nearly every pollen grain had germinated; some had quite long tubes, while others had shorter tubes; (*b*) almost every pollen grain had germinated and formed a very long tube; (*c*) many had germinated and formed short tubes; (*d*) a considerable number had germinated and formed short tubes; (*e*) and (*f*) same as (*d*); (*g*) none had germinated; (*h*) none had germinated and very few of the pollen grains had swelled.

B_1.—Outside cultures: 1:00 P.M., May 7; temperature 10°.5 C.: (*a*) many had produced long pollen tubes, while others showed no signs of germination; the sugar solution was partly evaporated and therefore was stronger than 5 per cent; (*b*) a very few had

germinated and the growth was much less than in (*a*); considerable evaporation had taken place; (*c*) a considerable number had germinated but the growth was less than in (*a*); some evaporation had taken place; (*d*) some had germinated and formed fairly long tubes; some evaporation had taken place; (*e*) a very few had formed short pollen tubes; very little evaporation; (*f*) same as (*e*); (*g*) only one pollen tube observed; evaporation very slight; (*h*) none had germinated; evaporation very slight.

A_2.—Inside cultures: 10:15 A.M., May 8; temperature 15° C.: (*a*) some had produced very long tubes and appeared to have almost exhausted the reserves in the pollen grain; there were a few which did not germinate; (*b*) same as (*a*); (*c*) pollen tubes mostly short; a very considerable number had not germinated at all; (*d*) these had formed much longer pollen tubes than in (*c*); the pollen grains were larger than those in (*c*) and may have been obtained from a different variety; some did not germinate; (*e*) same as (*d*); (*f*) many had formed comparatively long pollen tubes, although a considerable percentage did not germinate; (*g*) a number had formed pollen tubes of variable length, although the majority did not germinate; (*h*) three were observed with short pollen tubes.

B_2.—Outside cultures: 11:00 A.M., May 8; temperature 9° C.; temperature at 3:00 P.M., when they were examined, was 11° C.: (*a*), (*b*), and (*c*) same as on previous day; (*d*) a considerable number had formed short pollen tubes, but many had not germinated; (*e*) and (*f*) same as on previous day; (*g*) an extremely small number had formed short pollen tubes; (*h*) none of the pollen grains had germinated and very few of them had swelled.

A_3.—Inside cultures: 3:35 P.M., May 9; temperature 18° C.: (*a*) same as on previous day; (*b*) a considerable number had not yet germinated; (*c*), (*d*), (*e*), (*f*), and (*g*) same as on previous day; (*h*) a considerable number had formed fairly long pollen tubes.

B_3.—Outside cultures: 3:55 P.M., May 9; temperature 12° C.: (*a*), (*b*), and (*c*) same as on previous day; (*d*) a considerable number had formed pollen tubes of moderate length; (*e*), (*f*), (*g*), and (*h*), same as on previous day.

In all cases the pollen grains appeared to have 3 germ pores.

SERIES II

May 8, 1913.—A single flower of Bismarck apple was used for this set of experiments. It rained the whole day. Of the 20 stamens in the flower 13 had dehisced and all the pollen was washed off by the rain. Of the remaining 7, 5 were kept in a corked bottle over night and 2 were teased in 5 per cent and 10 per cent sugar solution respectively, and the cultures were placed outside in the garden at 9:30 P.M., the temperature being 4°.5 C.; the minimum temperature during the night was 3°.5 C., and the temperature on the following morning (May 9) at 8:50 A.M. was 7° C. The cultures were examined at 9:30 A.M. A few of those in the 5 per cent sugar solution had formed pollen tubes, but the majority had not germinated. In the 10 per cent solution one pollen grain was observed with a very short pollen tube.

May 9, 1913.—Three of the 5 stamens kept over night were teased in (*a*) water, (*b*) 5 per cent sugar, and (*c*) 10 per cent sugar solution respectively, and the cultures were placed at 12:15 P.M. in an incubator at a temperature of 20–21° C. The other two stamens were teased in (*d*) 5 per cent and (*e*) 10 per cent sugar solution respectively, and the cultures were placed on the window ledge outside the laboratory at 12:15 P.M. The temperature outside the window at 3:00 P.M. was 12°.5 C. The 5 cultures were examined at 4:15 P.M. on the same day, with the following results: (*a*) some of the pollen grains had formed tubes of considerable length; (*b*) several pollen grains had formed short pollen tubes; (*c*) a few pollen grains had germinated and in some cases the pollen tubes were of considerable length; (*d*) a number of pollen grains had formed short pollen tubes; (*e*) only a very few had formed pollen tubes of moderate length.

SERIES III

May 13, 1913.—Six cultures of pollen of Bramley's seedling apple taken from the same anther were prepared, namely, (*a*) in water, (*b*) 2.5, (*c*) 5, (*d*) 10, (*e*) 15, and (*f*) 20 per cent sugar solution respectively. The pollen grains were collected on May 10, 1913, and were kept dry. The cultures were started at 4:30 P.M.

on May 13, the temperature being 17° C., and they were kept in the dark.[1]

May 14, 1913.—The cultures were examined at 12:20 P.M., the temperature being 16°.5 C.: (*a*) very few had germinated; one had a pollen tube 58.5 μ long and another a pollen tube 309 μ long between perpendicular lines; (*b*) very few had germinated; one had a pollen tube 167 μ long; (*c*) very few had germinated; one had a pollen tube 150.3 μ long; (*d*) very few had germinated; one had a pollen tube 58.5 μ long and another a pollen tube 108.5 μ long; (*e*) no pollen tubes observed; (*f*) only a few had formed short pollen tubes, which were covered with short knoblike excrescences and did not seem to be healthy.

May 15, 1913.—The temperature at 4:00 P.M. was 16° C.; the results of examination were as follows: (*a*) an extremely small percentage had germinated; (*b*) no change; the culture was overrun with yeastlike cells of *Penicillium;* (*c*) and *d*) same as (*b*); (*e*) two pollen tubes observed; *Penicillium* plentiful; (*f*) no change; *Penicillium* abundant.

SERIES IV

May 15, 1913.—A flower of Warner's King apple was pulled this morning. On examination some of the pollen grains showed short pollen tubes which had been formed inside the anther. Five cultures of the pollen grains were made, namely, (*a*) in water, (*b*) 2.5, (*c*) 5, (*d*) 10, and (*e*) 15 per cent sugar solution respectively. They were put into an incubator at 22.5–23° C. at 10:40 A.M. the same day and examined at 4:35 P.M. The results were as follows: (*a*) most of the pollen grains had formed long pollen tubes; one measured 451 μ and another 384 μ, and these were about the average lengths; (*b*) almost every pollen grain had produced a long tube; the average length of the pollen tubes was 634.6 μ; (*c*) only a few had germinated and the pollen tubes were of short, irregular, tuberculate growth; (*d*) only a few had formed pollen tubes of irregular growth; (*e*) two or three pollen grains had formed very short pollen tubes of irregular growth.

[1] In measuring the length of pollen tubes in this and subsequent series, as the course of the pollen tube was usually very tortuous, it was found much more convenient to measure the distance between two lines drawn at right angles to the general direction of the pollen tube.

SERIES V

May 19, 1913.—Pollen grains of Bramley's seedling apple collected on May 10, 1913, and kept dry, were started in the laboratory at 4:45 P.M. on May 19, 1913. The temperature was 14°.5 C. Six cultures were made, namely, (*a*) water, (*b*) 1, (*c*) 2, (*d*) 4, (*e*) 8, and (*f*) 16 per cent solution of cane sugar. They were examined on May 20 at 3:30 P.M., when the temperature was 14°.5 C. and the results were as follows: (*a*) only a few had germinated; one had a pollen tube 150.3 μ between perpendiculars and there were others still longer; (*b*) only two short pollen tubes observed; (*c*) a few had formed short pollen tubes; (*d*) only a very few had formed short pollen tubes; the longest seen was 81 μ long and had a cauliflower-like growth at the end; (*e*) a small number had formed short pollen tubes; (*b*) an extremely small percentage had formed short pollen tubes.

On May 22 at 3:50 P.M., the temperature being 14°.5 C., the cultures were again examined, with the following results: (*a*) only a few had germinated, but they had formed fairly long pollen tubes; one measured 300.6 μ; (*b*) a very few had formed short pollen tubes; (*c*), (*d*), (*e*), and (*f*) same as on May 20; an abundant growth of *Penicillium* had occurred in these four. This series of cultures was kept continually in the dark.

SERIES VI

May 20, 1913.—Pollen grains of Bramley's seedling apple which were collected on May 10 and kept dry were put into the incubator at 22° C. at 10:10 A.M. Six cultures were made, namely, (*a*) water, (*b*) 1, (*c*) 2, (*d*) 4, (*e*) 8, (*f*) 16 per cent sugar solution. The results at 4:10 P.M. the same day were as follows: (*a*) and (*b*) none had germinated; (*c*) one pollen tube was 250.5 μ long, another 384 μ long; several others with pollen tubes of various lengths were observed, but only a small percentage altogether had germinated; (*d*) only a very few had formed short pollen tubes; (*e*) a fair percentage had formed long pollen tubes, one being 651.3 μ long; (*f*) a considerable number had formed pollen tubes, one being 200.4 μ long.

The results on May 21 were as follows: (*a*) two pollen tubes were observed but they did not seem to be healthy; (*b*) one pollen

tube, 300.6 μ long, was seen; hardly any other pollen grains had germinated; (*c*) only a very small number altogether had germinated; (*d*) no change; (*e*) a considerable number had formed long pollen tubes, the average length being 1336 μ; (*f*) a considerable number had formed pollen tubes of medium length, but the results were not so good as in (*e*).

SERIES VII

June 5, 1913.—Pollen grains of Warner's King apple collected on May 10, 1913, were put into the incubator at 21° C. at 4:15 P.M. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. The cultures were examined at 12:45 P.M. on June 6, with the following results: (*a*) a considerable number had formed short pollen tubes, some being 50.1 μ long; (*b*) many had germinated, forming pollen tubes 200.4 μ long; some were as much as 317.3 μ long; (*c*) many had germinated, some of the pollen tubes being 300.6 μ long.

SERIES VIII

June 16, 1913.—Pollen grains of Cox's orange pippin, which were collected on May 10 and kept dry, were started in the laboratory at 4:10 P.M., the temperature being 17° C. Three cultures were made, namely, (*a*) 8, (*b*) 16, and (*c*) 20 per cent sugar solution. They were examined at 12:45 P.M. on June 17, the temperature being 18° C., with the following results: (*a*) and (*b*) a few short pollen tubes were observed in each; (*c*) the pollen tubes were considerably longer than in (*a*) and (*b*). The cultures were again examined at 10:30 A.M. on June 19, the temperature being 16° C., but no further development of pollen tubes had taken place.

SERIES IX

June 26, 1913.—Pollen grains of Bismarck apple collected on May 10 were started in the laboratory at 4:55 P.M., the temperature being 16°.5 C. Three cultures were made in (*a*) 16, (*b*) 20, and (*c*) 40 per cent sugar solution. The cultures were examined at 12:10 P.M. on June 27, the temperature being 16° C., with the

following results: (*a*) two long pollen tubes were observed; (*b*) a few were beginning to germinate; (*c*) no pollen tubes were observed.

On June 28 at 10:35 A.M., the temperature being 16°.5 C., the results were as follows: (*a*) a few more were beginning to germinate; (*b*) a few had formed pollen tubes; (*c*) no pollen tubes were observed. On June 30 at 10:30 A.M., the temperature being 18° C., no further growth in any of the cultures had taken place.

SERIES X

August 6, 1913.—Pollen grains of several varieties of apple which were collected on May 10 and kept dry were started at 5:00 P.M., the temperature of the laboratory being 16° C. The following were the varieties and strengths of sugar solution used: (*a*) Nelson 4 per cent, (*b*) Wyken pippin 16 per cent, (*c*) Warner's King 20 per cent, (*d*) Bramley's seedling 4 per cent, (*e*) Duchess of Oldenburgh 16 per cent, (*f*) Bismarck 20 per cent, (*g*) Cox's orange pippin 16 per cent. The cultures were examined at 4:45 P.M. on August 7, the temperature being 16° C. The results were as follows: (*a*) two short pollen tubes were observed; (*b*) and (*c*) no pollen tubes; (*d*) several short pollen tubes; (*e*), (*f*), and (*g*) no pollen tubes.

They were examined again at 5:00 P.M. on August 8, the temperature being 16° C., with the following results: (*a*), (*b*), (*c*), (*d*), and (*e*) no change; (*f*) a considerable number of short pollen tubes were seen; (*g*) no change.

Pear

The variety used was Doyenne du Comice. The dry pollen grains were elliptical and measured 42×25.5 μ.

SERIES I

May 29, 1913.—Pollen grains which were collected on May 27 were started at 11:05 A.M. in the incubator at 21° C. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined the same day at 4:40 P.M., with the following results: (*a*) many had germinated, some of the pollen tubes being 551.1 μ long between perpendiculars; (*b*) several had

formed pollen tubes 617.9 μ long; (*c*) many had germinated, but the pollen tubes were short, being 133.6 μ or less in length.

SERIES II

June 12, 1913.—Pollen grains which were collected on May 27 and were started at 3:50 P.M. in the laboratory, the temperature being 15° C. As in all other experiments, they had been kept dry since the date of collecting. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 4:20 P.M. on June 13, the temperature being 15° C. The results were as follows: (*a*) many had germinated, some of the pollen tubes being 601.2 μ long; (*b*) many had germinated, the growth being better than in (*a*); some of the pollen tubes were 701.4 μ long; (*c*) many had germinated, some of the pollen tubes being 551.1 μ long.

SERIES III

August 6, 1913.—The cultures were started at 5:00 P.M., the temperature in the laboratory being 16° C. The pollen grains had been collected on May 27, 1913. They were in 4 per cent sugar solution and were examined at 4:45 P.M. on August 7, the temperature being 16° C., but none had germinated. On examination at 5:00 P.M. on August 8, the temperature being 16° C., it was found that a few short pollen tubes had been formed.

Strawberry

SERIES I

An open flower was plucked on May 27, 1913, the weather having been dry for several days. The flower was kept dry until May 29, but still the anthers did not open. On teasing the anther on a slide no pollen grains escaped, but when the anther was teased in water small pollen grains were found which were subglobular in shape, but more or less shriveled and about 18 μ in diameter. Cultures in 4, 8, and 16 per cent sugar solution were started at 11:05 A.M. on May 29 in an incubator at 21° C., but at 4:40 P.M. none had germinated.

SERIES II

June 2, 1913.—Pollen grains from a different variety were collected on this date. When dry these were elliptical in shape, measuring $37.5 \times 19.5\ \mu$. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were started at 4:45 P.M. the same day, the temperature of the laboratory being 16° C. They were examined at 9:45 A.M. on June 4, the temperature being 15°.5 C., with the following results: (*a*) only a few had produced pollen tubes of medium length, and of these several were tuberculate at the tip; (*b*) and (*c*) similar to (*a*).

They were examined again at 11:00 A.M. on June 5, the temperature being 15°.5 C., with the following results: (*a*) nearly all of the pollen tubes formed were short and of irregular growth, and many of the pollen grains were small and apparently rudimentary; the longest pollen tube seen measured 267.2 μ; (*b*) the pollen tubes were a little more regular in their growth than in (*a*); one measured 250.5 μ; (*c*) similar to (*a*); a pollen tube measured 300.6 μ.

SERIES III

June 6, 1913.—Pollen grains were collected from a seedling strawberry plant on this date. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. The cultures were started at 4:00 P.M. in the laboratory, the temperature being 15°.5 C. They were examined at 3:50 P.M. on June 9, the temperature being 14°.5 C., with the following results: (*a*) no proper healthy pollen tubes were observed; (*b*) numerous long pollen tubes were observed, one measuring 1169 μ; (*c*) two short pollen tubes were seen, but the vast majority had behaved like those in (*a*).

SERIES IV

Pollen grains were collected on June 2, 1913, and started in 10 per cent sugar solution at 5:00 P.M. on August 6, the temperature being 16° C. At 4:45 P.M. on August 7 none had germinated. At 5:00 P.M. on August 8 the result was the same.

Loganberry

Dry pollen grains elliptical, $43.5 \times 22.5\ \mu$. June 2, 1913. Pollen grains were collected on this date and started in the laboratory at

4:45 P.M., the temperature being 16° C. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 9:45 A.M. on June 4, the temperature being 15°.5 C., with the following results: (*a*) many had germinated and formed long pollen tubes; (*b*) most of the pollen grains had attempted to germinate, but the pollen tube, as a rule, was short, being no longer than the diameter of the pollen grain, and irregular and tuberculate. A few had formed fairly long, properly developed tubes; (*c*) same as (*b*).

At 10:45 A.M. on June 5, the temperature being 15°.5 C., they were again examined: (*a*) a pollen tube measured 1052.1 μ long; (*b*) these had made little further growth from the previous day; a pollen tube measured 250.5 μ; (*c*) similar to (*b*) for the most part; a pollen tube was 467.6 μ.

An attempt was made on August 6 to germinate some of the pollen grains collected on June 2 in 4 per cent sugar solution, but no pollen tubes developed.

Raspberry

Dry pollen grains elliptical, 33×21 μ, globular when wet. On June 5, 1913, pollen grains were collected and put into the incubator at 21° C. at 4:15 P.M. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 12:45 P.M. on June 6, with the following results: (*a*) contents of the pollen grain had protruded slightly, but no proper pollen tubes were observed; (*b*) mostly similar to (*a*), but in a very few cases fairly well developed pollen tubes were found; (*c*) a considerable number had formed pollen tubes, one of which measured 317.3 μ between perpendiculars. An attempt was made on August 6 to germinate some of the pollen grains collected on June 5 in 16 per cent sugar solution, but without success.

Black currant

Pollen grains when dry spherical, 30 μ in diameter, in water spherical, 42 μ in diameter, with a number of germ pores.

SERIES I

May 22, 1913.—Pollen grains which were collected on May 19 were put into the incubator at 22° C. at 10:25 A.M. Four cultures were made, namely, (*a*) water, (*b*) 4, (*c*) 8, and (*d*) 16 per cent sugar solution. They were examined at 4:20 P.M. the same day, with the following results: (*a*) a small percentage had germinated, the average length of the pollen tube being 66.8 μ; (*b*) a large number had formed pollen tubes which were fairly straight, the average length being 250.5 μ; one measured 317.3 μ; (*c*) a large number had formed long pollen tubes, the average length being 634.6 μ; (*d*) a large number had germinated and formed pollen tubes 668 μ long or more.

SERIES II

June 6, 1913.—Pollen grains which were collected on May 19, were started in 8 per cent sugar solution at 4:00 P.M., the temperature of the laboratory being 15°.5 C. They were examined at 3:50 P.M. on June 9, the temperature being 14°.5 C. Some had germinated, one pollen tube being 434°.2 μ long.

SERIES III

Pollen grains collected on May 19 were tested in 16 per cent sugar on August 6, 1913, being examined on each of the two following days, but none had germinated.

Discussion

As regards the most suitable medium for germinating pollen grains, there is great diversity shown by different species of plants. Some pollen grains when immersed in ordinary tap water swell up and burst. This happens in *Geranium sanguineum*, *Convolvulus arvensis*, *Valeriana officinalis*, and *Scabiosa succisa*. MARTIN (5) states that the pollen of *Trifolium pratense*, *T. hybridum*, and *T. repens* bursts almost instantly when dropped into water. He found that the same thing happened in various sugar solutions. His results obtained in germinating the pollen grains of *Trifolium* were not at all uniform. He found that they germinated best on wet parchment paper or hog's bladder, the amount of moisture

present having an effect on germination. He further adds that microchemical tests of the papillae on the stigma showed no sugar or starch present, but an oily emulsion such as was found in the pollen. His general conclusions are as follows:

> From these observations it appears that the stigma secretes nothing that has any effect on germination or the direction of the pollen tube. The behavior of the stigma in the experiments at least indicates that its function in the germination of the pollen is to regulate the water supply.

Confirmation of these results is very much to be desired, as the experience of other workers in the case of various other species of plants is quite contradictory. The stigma in most plants has a secretory function; the amount of this secretion and its chemical composition will very probably vary to some extent with the amount of water absorbed by the roots, and with the relative humidity of the atmosphere, but from the morphological structure of the style and stigma it is extremely improbable that there is any special mechanism for controlling the water supply.

JOST (2) germinated pollen grains on starch paste made with only one or two parts of water, and also on parchment paper soaked with a sugar solution. This, it must be admitted, seems a more natural method of germinating the grains than immersion in a liquid medium, and resembles closely the condition found on the surface of the stigma. It has the drawback, however, of being more difficult to observe under the microscope. His general conclusions are as follows. He finds that germinating pollen grains may be placed in three classes: (1) those requiring nothing but water for germination, much mineral matter being injurious; to this group belong grasses, which can only germinate in minute quantities of pure water; (2) those requiring a very dilute solution of a definite chemical substance which is contained in the stigma; in a few cases this substance is levulose, in others organic acids, but in most cases it is unknown; (3) those which germinate only in a sugar solution of definite concentration.

According to PFEFFER (1), certain pollen grains will germinate only in the stigmatic fluid. MOLISCH (1) ascribes the curving of the pollen tube to the stigma to a chemotropic reaction. He adds further that pollen tubes curve toward water poorer in oxygen.

In my experiments, on the other hand, I found that absence of oxygen prevented the germination of the pollen grains. MIYOSHI (1) found that cane sugar, grape sugar, and dextrin exerted especially strong chemotropic attraction on pollen tubes, and that the first penetration of the stigma by the pollen tube was induced by chemotropic stimulation aided by the hydrotropism of the pollen tube, and possibly also by aerotropic and other stimuli.

SANDSTEN (3) carried out an extensive series of experiments with pollen grains. He found starch, diastase, and invertase in all the pollen grains which he examined. He also found diastase and invertase in the tissues of the style and stigma. He used hanging drop cultures of saccharose in almost every case. For the species of plants that he experimented with he found the optimum strength of sugar solution to lie between 5 and 35 per cent, but 20 per cent was the rule in the majority of instances. He found the range of concentration of the cane sugar for any given species of pollen to be large, indicating differences of degree of the concentration of the juices of the stigma. For instance, pollen of *Narcissus Tazetta* germinated in 1 per cent solution of cane sugar and also in a 60 per cent solution. He says that bursting of pollen takes place in masses of apple and plum pollen during warm spring rains, while in distilled water the contents protruded a distance equal to the diameter of the pollen grain, but made no further growth. I found a somewhat similar protrusion of contents in some of the sugar solutions used, but should hesitate to regard this as true germination. SANDSTEN further states that most pollen grains are negatively aerotropic and chemotropic, and that the direction of growth of the pollen tube is away from the light. Elsewhere he states that sunshine had little or no effect on the germination of the pollen or upon the growth of the pollen tube in most plants. In my experiments I found no difference in germination in the case of pollen grains kept in total darkness as compared with others exposed alternately to darkness and light.

Regarding the relation of the pollen grains to temperature, the temperature of the laboratory in SANDSTEN'S experiments varied from 15 to 36° C. He found the optimum degree of temperature for the germination of apple, pear, and plum pollen to be 24° C.

At 20° C. the average rate of growth of the pollen tube of apple was 280 μ for the first hour and 420 μ for the second hour. These rates of growth were much more rapid than any observed by me, but the optimum temperature agrees with my results, although 23° C. was the highest that I experimented with. SANDSTEN found that after exposure to a temperature of −1°.5 C. for 6 hours there was only a slight falling off in the germination of apple, pear, and plum pollen, while in cherry and peach the falling off in the germination was much more marked.

CHANDLER (**6**) exposed the pollen of Jonathan apple to a temperature of −3° C. for 18 hours and found that in a 10 per cent solution of sugar it gave a germination of 33 per cent. After exposure of dried pollen of the same variety to a temperature of −13° C. for 18 hours it gave a germination of 20 per cent. SANDSTEN found that exposure of the stigmas of the five species mentioned above to a temperature of −1°.5 C. for 6 hours caused the death of almost all of them.

Finally, as regards the length of time during which pollen grains can retain their vitality, SANDSTEN states that a small percentage of apple pollen retained its vitality for 6 months, while but few pollen grains of plum retained their germinating powers for this length of time. Both were kept dry at a temperature ranging between 7 and 26° C. In my experiments a few pollen grains of apple germinated after nearly 3 months, and of pear after 2 months, but no tests were made after longer intervals than these. It is very probable that SANDSTEN'S definition of what constitutes germination was different from mine. CRANDALL (**4**), taking fruit setting as the basis for determining the vitality of pollen, found that the maximum age at which pollen was successfully used was 11 days for apple and about 16 days for strawberry.

Summary

1. The following species were used for experiment: apple, pear, strawberry, loganberry, raspberry, black currant.

2. The culture medium was cane sugar and the strengths that gave the best germination were as follows: apple 2.5–10 per cent, pear 4–8 per cent, strawberry 8 per cent, loganberry 4 per cent, raspberry 16 per cent, black currant 16 per cent.

3. Some pollen grains of apple germinated in tap water and also in various strengths of sugar solution up to 50 per cent; the pollen grains of black currant germinated in tap water and also in 4, 8, and 16 per cent sugar solution.

4. Some pollen grains of apple germinated in 12 hours, the temperature ranging between 3°.5 and 7° C.

5. The quickest growth of the pollen tube observed was 651.3 μ in 6 hours in apple, and 668 μ in 6 hours in black currant, as measured between perpendicular lines.

6. Some varieties of the same species appeared to have more vigorous pollen grains than others.

7. The pollen grains germinated alike in light and darkness.

8. Of the temperatures employed, 21–23° C. gave the quickest germination.

9. A few pollen grains of apple formed short pollen tubes after being kept dry for about 3 months, and of pear after 10 weeks. The pollen grains of strawberry, loganberry, and raspberry were all dead after 2 months, and of black currant after 11 weeks.

CENTRAL EXPERIMENTAL FARM
OTTAWA, CANADA

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